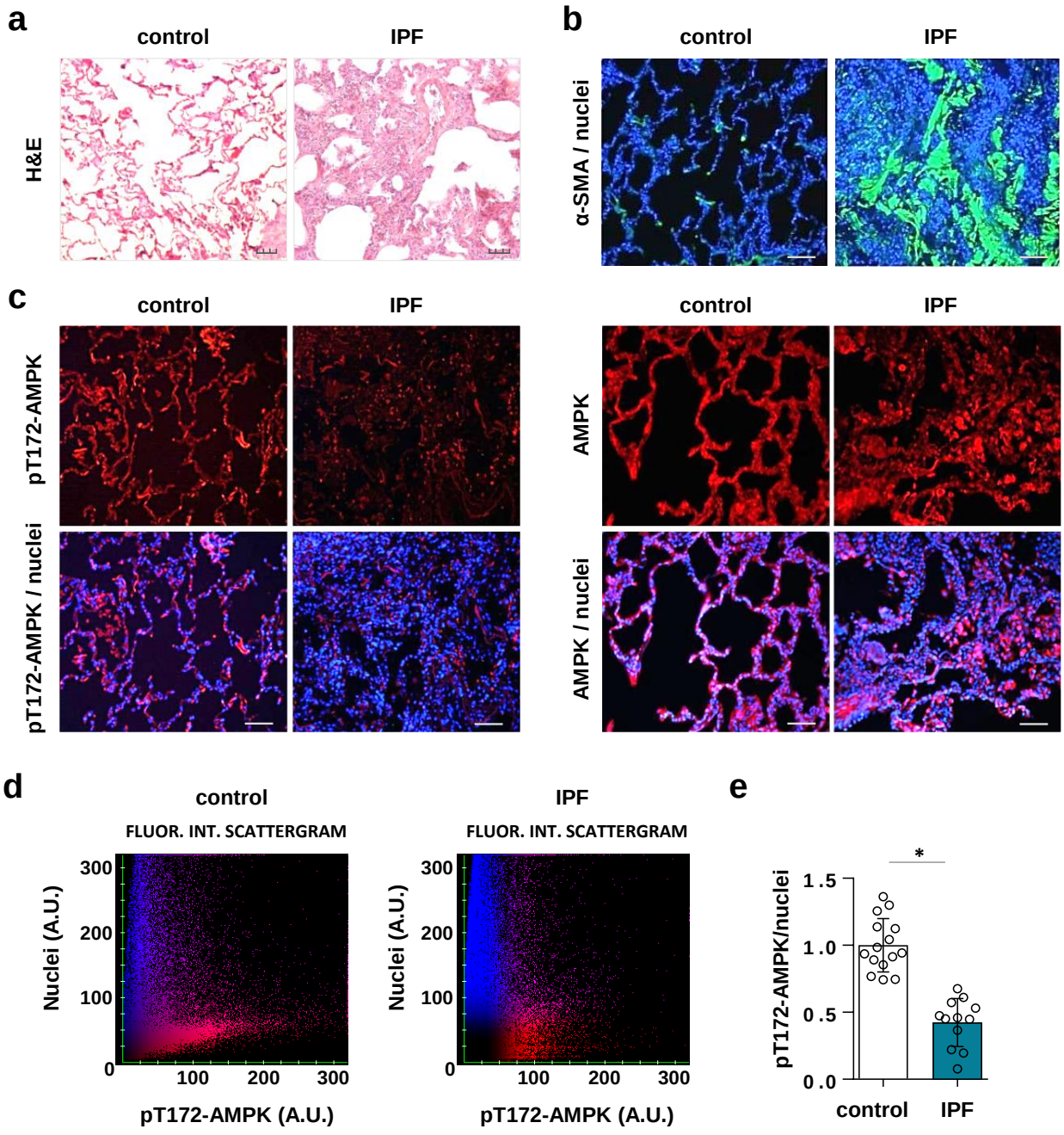
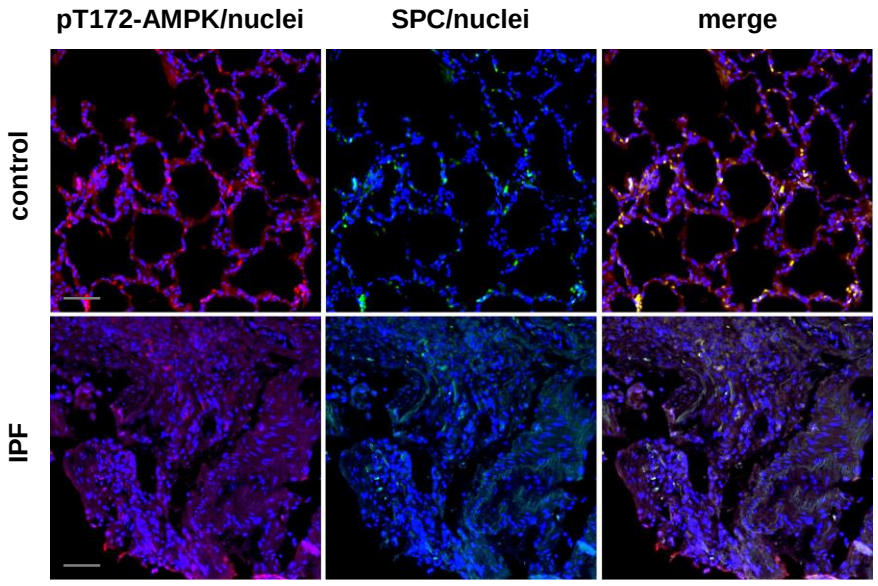
Supplemental Figure S1



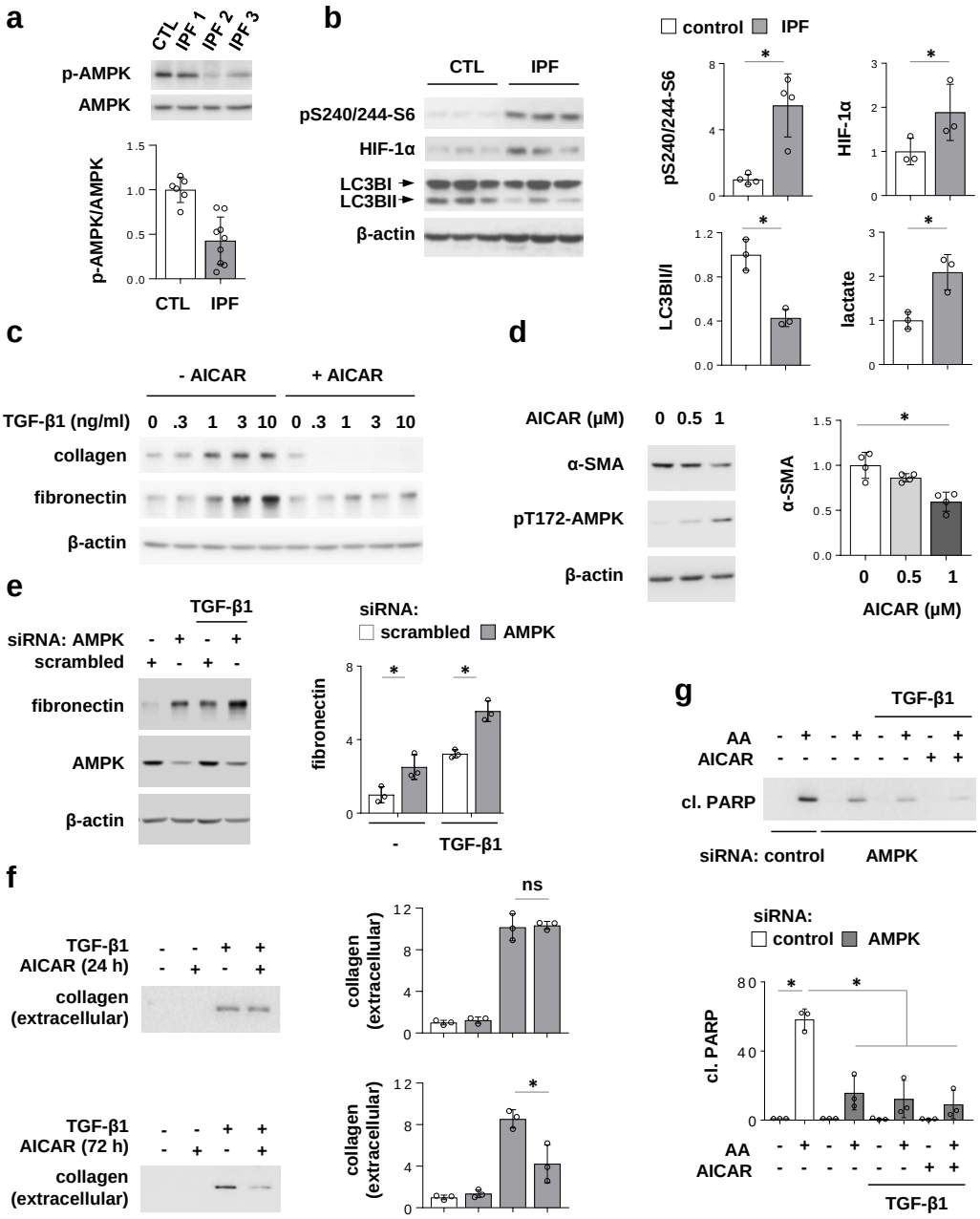
Supplemental Figure S1. AMPK activity is diminished in lung fibrotic regions from individuals with IPF. Representative images show (**a**) H&E, (**b**) α-SMA and (**c**) pT172-AMPK and total AMPK fluorescence in lung sections of control and IPF. α-SMA (green), AMPK (red), nuclei (blue). Scale bar 100 μm. (**d**) Representative scattergrams indicate pT172-AMPK (red) and nuclei (blue) fluorescence intensity (A.U.; arbitrary units). (**e**) pT172-AMPK/nuclei fluorescence ratio in randomly selected areas of lung sections from control and IPF. Means ± SD, n = 15 (control), n = 12 (IPF). * $P < 0.05$ (Student's t-test).

Supplemental Figure S2



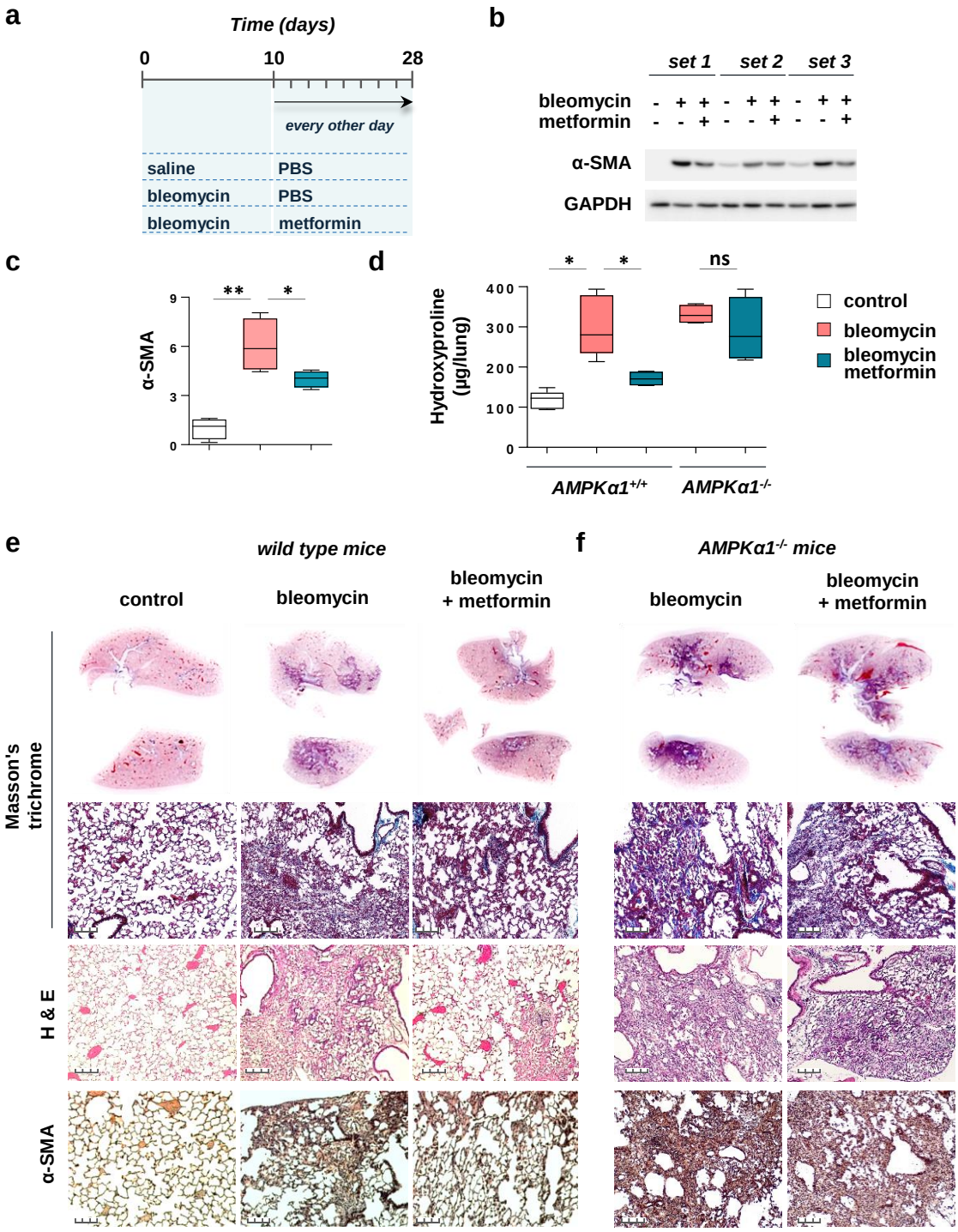
Supplemental Figure S2. Alveolar epithelial cells (Type II) and AMPK phosphorylation in lung sections of control and individuals with IPF. Representative images show p172-AMPK (red), epithelial marker SPC (Type II AECs; green), and nuclei (blue) fluorescence in lung sections from control and subject with IPF. Scale bar 100 μm.

Supplemental Figure S3



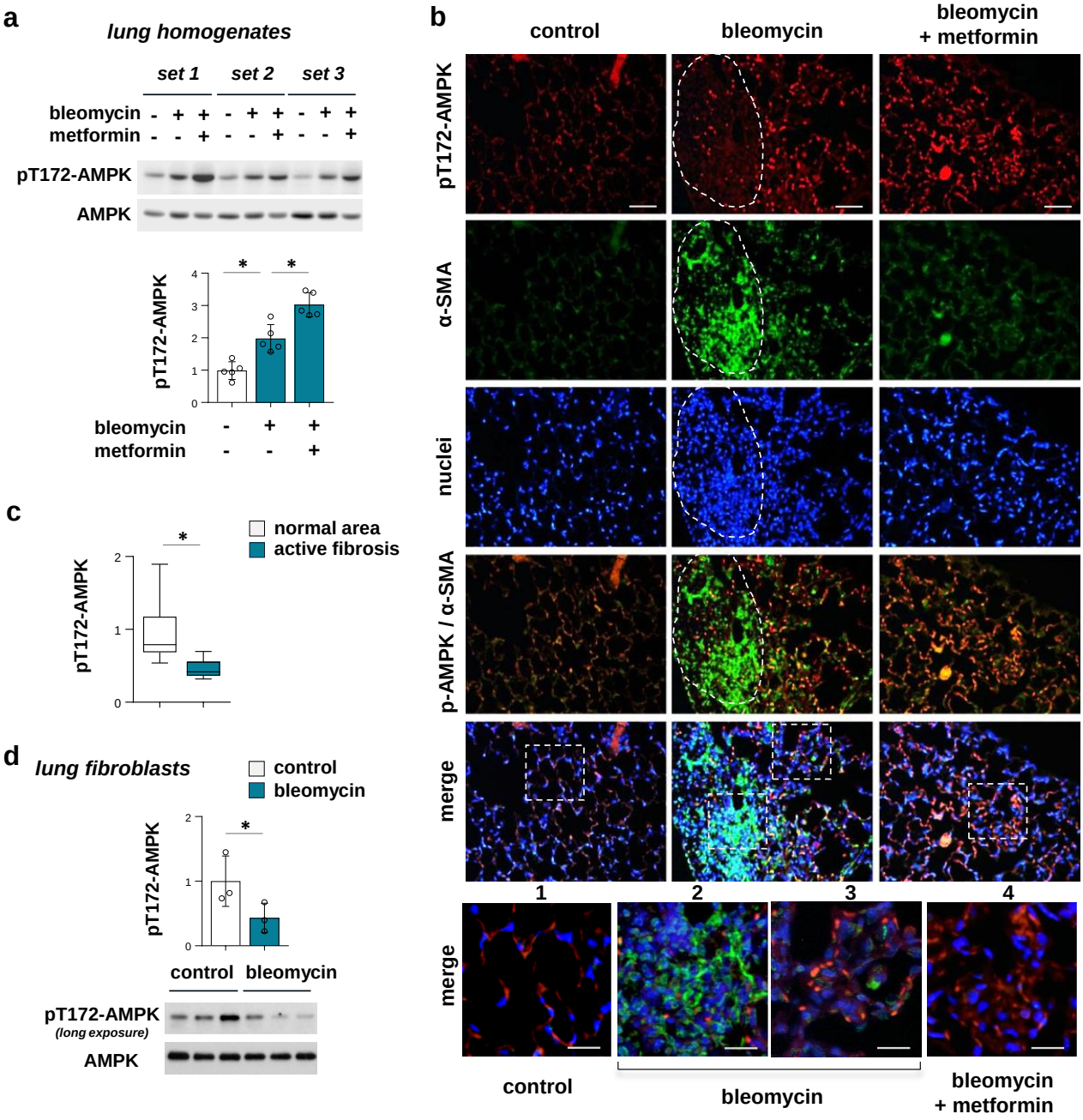
Supplemental Figure S3. Effects of AMPK activation on ECM protein expression and resistance to apoptosis in lung fibroblasts. (**a**) Representative western blots indicate pT172-AMPK and total AMPK from control and IPF fibroblasts. Means ± SD, $n = 6$ (controls), $n = 9$ (IPF). *$P < 0.05$ (Student's t-test). (**b**) Representative immunoblots indicate pS6, HIF-α1, LC3BI/II and β-actin in control and IPF fibroblasts. Means ± SD, $n = 3$ (controls) and $n = 3$ (IPF). *$P < 0.05$ (Student's t-test). (**c**) Representative immunoblots show collagen, fibronectin and β-actin in control lung fibroblasts treated with AICAR for 2 hours, followed by exposure to TGF-β1 in doze dependent manner (24 hours). (**d**) Representative immunoblots show pT172-AMPK, α-SMA and β-actin in fibroblasts treated with AICAR for 2 hours and than TGF-β1 (24 hours). Means ± SD, $n = 4$. *$P < 0.05$ (ANOVA). (**e**) The levels of fibronectin and β-actin in control (scrambled siRNA) and human lung fibroblasts with siRNA-mediated silencing of AMPK. Cells were treated with TGF-β1 for 24 hours, as indicated. Representative western blots are shown. Means ± SD, $n = 3$, *$P < 0.05$ (ANOVA). (**f**) Representative immunoblots show the levels of extracellular collagen accumulation in TGF-β1-treated human lung fibroblasts for 24 hours, followed by inclusion of AICAR for 24 or 72 hours. (**g**) Immunoblot of cleaved PARP in control (scrambled siRNA) or lung fibroblasts with siRNA-mediated silencing of AMPK. Cells were incubated with TGF-β1 for 24 hours followed by exposure to AICAR (500 μM; 3 days), and then antimycin A (AA; 16 hours). Representative western blot is shown (**f**,**g**) Means ± SD, $n = 3$. *$P <$ 0.05, ns-not significant (ANOVA).

Supplemental Figure S4



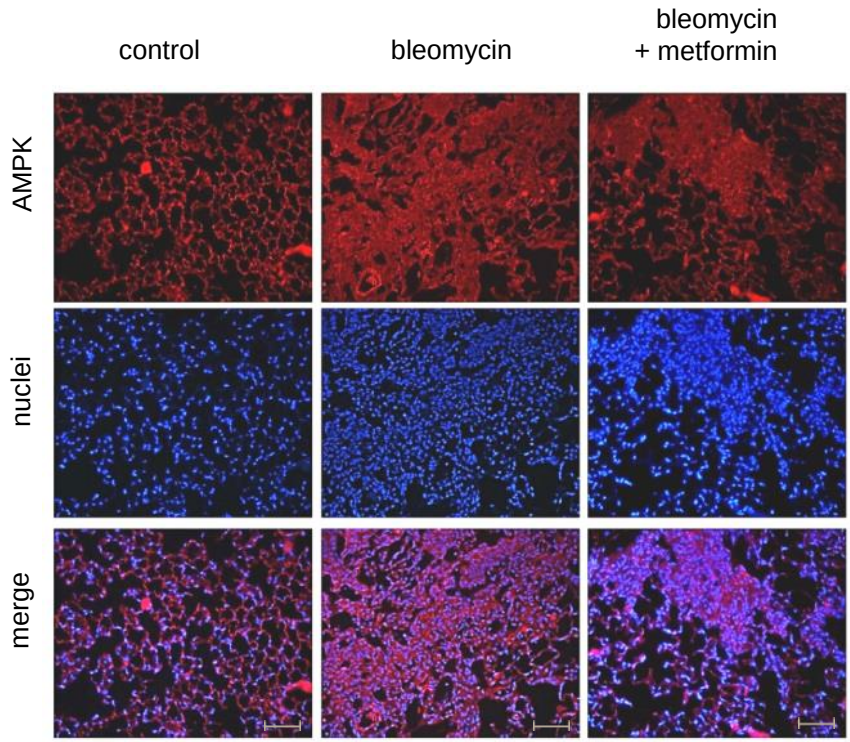
Supplemental Figure S4. Metformin reduces collagen accumulation during the fibrotic phase. Panel (**a**) outlines the design of therapeutic dosing of metformin in bleomycin-treated mice. (**b**,**c**) Representative western blots and optical bend densitometry of α-SMA and GAPDH in mouse lung homogenates from groups of mice indicated in **a**. Means ± SD, *n* = 4 mice per group. (**d**) The levels of hydroxyproline in lung homogenates from indicated groups of mice depicted in **a**. Means ± SD, *n* = 5 mice per group, * $P < 0.05$ (ANOVA). (**e**,**f**) Representative images display Masson's trichrome, H&E, and α-SMA staining (whole lung sections or selected areas) from control or mice treated with bleomycin or bleomycin and metformin; (**e**) wild type or (**f**) *AMPKα1*$^{-/-}$ mice. Scale bar 100 μm.

Supplemental Figure S5



Supplemental Figure S5. Differential pattern of AMPK activity in AECs and myofibroblast during the fibrotic phase. (**a**) Representative immunoblots of pT172-AMPK and total AMPK from lung homogenates of control, bleomycin, or mice treated with bleomycin and metformin, as described in **Supplemental Figure 4a**. Means ± SD, *n* = 5. **P* < 0.05 (ANOVA). (**b**) Representative images show pT172-AMPK fluorescence in the lung sections from indicated groups of mice. The elliptical lines (dashed) indicate region of active fibrosis, *i.e.* positive for α-SMA fluorescence. pT172-AMPK (red), α-SMA (green), nuclei (blue). Scale bar, 100 μm. Lower panel shows higher magnification of images from regions marked by square boxes (dash lines). Scale bar, 50 μm. (**c**) pT172-AMPK/nuclei fluorescence ratio in normal and fibrotic regions from bleomycin-treated mice. Means ± SD, 3 mice per group, *n* = 11 normal areas, *n* = 10 fibrotic regions. **P* < 0.05 (Student's *t*-test). (**d**) Representative immunoblots show pT172-AMPK and total AMPK in lung fibroblasts isolated from mice, *i.e.* 21 days after bleomycin-mediated lung injury. Means ± SD, *n* = 3 mice per group. **P* < 0.05 (Student's *t*-test).

Supplemental Figure S6



Supplemental Figure S6. The AMPK fluorescence in lung sections of control and mice treaded with bleomycin or bleomycin and metformin (treatment is depicted in **Supplemental Fig. 4a**). Representative images show total AMPK (red) and nuclei (blue) fluorescence. Scale bar 100 μm.

Supplemental Tables 1 and 2.

Table 1. Patient demographics

Lung sections					
#	Age	Sex	Race	Condition	Smoking status
2026	60	M	W	IPF	no
2032	66	M	W	IPF	no
2041	56	M	W	IPF	no
2058	58	M	AA	IPF	no
2059	61	F	W	IPF	no
LTRC 168352	61	M	W	IPF	past Smoker
LTRC 168219	76	M	W	IPF	past Smoker
LTRC 102695	59	F	W	IPF	past Smoker
LTRC 277811	82	M	W	IPF	past Smoker
3013	61	F	W	normal	no
3008	49	M	W	normal	no
3007	55	M	W	normal	no
3014	45	F	W	normal	no
3004	53	F	W	failed donor	no
LTRC 045399	76	M	W	normal	no
LTRC 141224	79	F	W	normal	no
LTRC 286902	51	F	W	normal	past smoker
LTRC 120371	60	M	W	normal	past smoker

Table 2. Patient demographics

Lung fibroblasts					
#	Age	Sex	Race	Condition	Smoking status
15044	53	M	W	IPF	past smoker
15061	63	F	W	IPF	past smoker
2041	56	M	W	IPF	no
2032	66	M	W	IPF	no
2617	63	M	W	IPF	no
15046	65	M	AA	adenocarcinoma*	past smoker
15050	49	M	W	adenocarcinoma*	past smoker
16023	52	M	W	adenocarcinoma*	no
16029	69	M	W	pneumonia**	smoker
16031	61	F	A	adenocarcinoma*	no

* normal lung tissue resected from adenocarcinoma
** normal lung tissue resected from pneumonia